

Hiding in Plain Sight: A Consideration of NF1-Associated Hypovitaminosis D and its Treatment

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Abstract

This article deals with the autosomal dominant human genetic disorder, Neurofibromatosis Type 1 (NF1), and has three main foci. The first is the general principle that substantial advances in understanding the pathogenesis of a genetic disorder can derive from the timely reconsideration of material previously overlooked or otherwise not given its due. There are times when the key to a supposed "mystery" is hiding in plain sight. The second focus is the specific consideration of selected elements of an apparent vitamin D deficiency in NF1. And the third focus of this article reconsiders the mast cell in NF1 pathogenesis, including the potential of NF1 being a "mastocytosis" of sorts, with the prospect of additional grounds for treatment with mast cell blockers, for example, ketotifen.

Keywords: Hypovitaminosis D; Vitamin; NF1 mastocytosis

Introduction

The primary purpose of this article is to consider several sets of observations relevant to the autosomal dominant human genetic disorder, Neurofibromatosis Type 1 (NF1; OMIM 162200) and to suggest that they have been inadequately followed up. This inadequacy reflects the current era of emphasis – almost exclusively – on mutations and clonality. In particular, I suggest an important tie-in of two of these "observations," namely NF1-associated hypovitaminosis D (hypocalcidiemia) and NF1 mastocytosis.

There have been at least five substantive associations that are not necessarily predictable from the original NF1 clinical phenotype or the details of the causative genotype, namely a mutation in or deletion of the NF1 gene locus on the long arm of human chromosome 17. These five items include 1) the amazingly consistent cell-specific and locus-specific somatic mutations (2nd hit) [1-3]; 2) blunt trauma and wound-healing dynamics as contributory to the origin and progression of neurofibromas; [4] 3) the under-representation of obesity and diabetes mellitus among persons with NF1 (unpublished data); 4) the pivotal role of mast cells in the initiation and persistence of neurofibromas; [5, 6] and 5) the documentation of low serum calcidiol levels among persons with NF1 [7]. A sixth consideration could easily be the high frequency and distinctive types of NF1 cardiovascular abnormalities. They include both congenital heart disease and cardiomyopathy and several types of developmental vascular dyscrasias, most often involving the carotid-vertebral-brachial artery complex, the renal arteries, the superior mesenteric artery and the mid-portion of the aorta. In particular, there is nothing we know about the NF1 gene locus per se [8] to suggest or explain abnormalities of vitamin D metabolism. Certainly, the most intensely studied NF1 domain-the GAP-related domain, or GRD-provides no intrinsic clue. Likewise, the nature of the widespread skeletal involvement in NF1 does not compel concern for hypocalcidiemia. Finally, there is naught about either mast cells or vitamin D metabolism themselves to associate them causally in terms of vitamin D deficiency.

This latter fact notwithstanding, respecting my long-term interest in mast cell contributions to NF1 pathogenesis, [5] I recently reviewed my 30-year accumulation of some 12,000 or more NF-related publications. This bioinformatics quest, seeking to identify links between NF1, mast cells and vitamin D, yielded immediate and substantial results: mammalian skin mast cells are critical determinants of the fate of substantial portions of skin-derived vitamin D. This is especially important in NF1 with its excesses of mast cells in both normal tissues and neurofibromas. Here I will both explore the relevant literature and synthesize the data to illuminate selected elements of NF1 pathogenesis and thereby entertain new-or renewed-therapeutic approaches.

Vitamin D Background

The keratinocytes (KC) of mammalian skin de novo synthesize 7-dehydrocholesterol, which is converted by these same cells to vitamin D₃, also known as calcidiol or cholecalciferol. According to the canonical accounting of mammalian vitamin D biochemistry, KC-derived calcidiol is transported by the blood to the liver, where it is converted to calcidiol (25-hydroxycholecalciferol). Calcidiol is then transported by the blood to the kidneys, where it is converted to calcitriol (1,25-dihydroxycholecalciferol). The latter is the active form of the vitamin D hormone and is the substance that interacts with the vitamin D receptor (VDR) in multiple tissues, including bone, cardiac striated muscle and skeletal striated muscle. Other physiological and biochemical moieties, such as "vitamin D-binding protein" may also be relevant ultimately, but will not be considered here.

The well-known dependence of KC production of calcidiol on ultraviolet wave length photons from sun exposure is a major factor in maintaining normal amounts of calcidiol and calcitriol in humans. This, in turn means that factors determining the duration and intensity of incident sunlight on human skin influence the availability of the various congeners of vitamin D. Geography, particularly extreme North and South latitudes, and clothing are strong determinants of how much sun impinges on human skin [7,9]. Dietary factors, including vitamin-D-fortified foods and multi-vitamin supplementation are also important factors. Now it also appears that

other cells and various genetic and environment-imposed disorders may influence the availability of calcidiol, calcitriol and calcitriol, although ultimately, it is the availability of calcitriol that is the key to this hormone's contribution to a person's health.

Vitamin D and NF1: Prologue

A 2001 article by Illes et al. [10] documented a "diminution of NF1 axial bone mineral density." They concluded that the analyzed "biochemical parameters do not support hyperparathyroidism, renal disorders or other diseases influencing the bone mineral turnover." However, vitamin D substances were not investigated. Three years later, Juha Peltonen's group elaborated on the skeletal dynamics of Nf1 mutant mice [11] and made no mention of vitamin D. After one more year, in 2004, Yu et al. [12] reported on bone abnormalities in Nf1 ± mice and commented on vitamin D only in terms of its availability in the food source, 4500 IU/Kg. In 2006, Lammert et al. [7] documented both a statistically significant low level of calcidiol in an NF1 population ($p=0.0001$) and an inverse correlation of serum calcidiol and the number of "dermal" (presumably cutaneous) neurofibromas ($p=0.00001$).

As considered more completely below, it is possible, if not likely, that the NF1 "neurofibroma burden" actually accounts for this hypocalcemia, essentially making the latter an "acquired condition." Consistent with this hypothesis, in 2014, Schnabel et al. [9] did not find hypocalcemia in 46 NF1 children (2-17 years of age) with no significant neurofibroma burden. The apparent influence of NF1 neurofibromas, of course, raises the possibility of avoiding the hypocalcemia by minimizing the size and number of NF1 neurofibromas. It is unlikely that merely adding vitamin D supplements will deal with the vitamin D problem at its root.

In 2008, in a study focusing on NF1 adults, Brunetti-Pierri et al. [13] showed that in NF1 "parathyroid hormone was modestly elevated and after four months of supplemental therapy with calcium and vitamin D, it decreased to the normal range. However, bone mineral density z-scores did not significantly improve after 2 years of follow-up." In addition, in 2014, Heerva et al. [14] studied six NF1 adults (28-76 years of age), three of whom had serum calcidiol levels less than 50 nmol/L. After 23 months of treatment with alendronate and vitamin D supplementation, there was no change in bone mineral density or serum levels of the osteoclasts marker, tartrate-resistant acid phosphatases (TRAP5b). There were, however, reductions in serum levels of the collagen type I markers C-terminal telopeptide (CTX) and N-terminal propeptide (PINP). In other words, neither vitamin D supplementation nor serum calcidiol levels tell the whole story.

Data consistent with low levels of serum calcidiol in at least some persons with NF1 date from about 2001. However, since low serum calcidiol among persons with NF1 appears to have been confirmed, for example, by Stevenson et al. [15] I am puzzled. One of humanity's most important nutrient hormones is deranged in one of humanity's most common genetic disorders and yet NF1 clinicians and investigators seem to settle for both an incomplete characterization of the "deficiency" (e.g., where do calcitriol and the VDR fit in?) and an incomplete exploration of the origin and ramifications of the "deficiency."

Adding to the conundrum is the fact that Armstrong et al. reported in 2013, in an 18-pair sibling-comparison study, [16] that "the mean values for serum vitamin D [calcidiol] in [NF1] cases and controls in our study and that of Brunetti-Pierri et al. from 2008 [13] are not

significantly different." A similar NF1-sibling comparison study drew the same conclusion [17]. Serum calcidiol concentrations were 67 and 62 nmol/L for cases and siblings, respectively. However, the vitamin D supplement was 344 and 314 IU/day for cases and siblings, respectively. These data are consistent with the finding that "All cases and controls had serum calcium concentrations within the normal range." However, do "normal serum levels of calcidiol" in the face of vitamin D dietary supplementation allow us to discount the earlier finding of NF1-associated low serum levels of calcidiol when vitamin D supplementation was a basis for exclusion from that latter study?

I think not-for two sets of reasons. First, the data of Lammert et al. [7] relate "normal" serum calcidiol levels with few or no cutaneous neurofibromas. Given the young ages of the Armstrong et al. NF1 subjects and sibling controls (13.8 and 14.0 years, respectively) and the apparent absence or paucity of cutaneous neurofibromas in the NF1 subjects, we should not be surprised that serum calcidiol levels are comparable in the two groups. (The term, "neurofibroma" was not used at all in the Armstrong et al. article, including the description of the NF1 subjects.) Similarly, Hockett et al. [17] showed no difference for 15 NF1 subjects and age-matched siblings in terms of serum calcidiol and serum calcitriol, although, again, comparable levels of vitamin D supplementation were documented for the two subsets. However, circulating levels of parathyroid hormone were higher in the NF1 subset ($p=0.05$). One could even conclude that both the Armstrong et al. and the Hockett et al. data substantiate the inverse relation of NF1 serum calcidiol and cutaneous neurofibromas. I would suggest that this is the correct conclusion and, as concluded by Stevenson et al. [15] age is an important variable. A second reason for not relying solely on low serum calcidiol in NF1 is that calcidiol is only one component in a very complex biochemical schema. That is, serum calcidiol levels may not be the best proxy for the schema in its entirety. On the one hand, serum calcidiol may not accurately reflect either calcidiol production or calcitriol blood levels or availability [18]. On the other hand, complementary data, such as blood and urine concentrations and/or daily production of histamine may be useful. There is something more at stake than simply blood levels of a single vitamin D congener, especially considering the multiplicity of ways the congeners are inter-related. The available data suggest that, in NF1 there is a disturbance in the parsing or compartmentalization of the various substances, specifically calcidiol, calcidiol and calcitriol. In this context, a pair of articles dealing with vitamin D in various mammals [19,20] seem to be especially cogent and, in particular, are consistent with the inverse relationship of NF1 serum calcidiol and cutaneous neurofibromas.

Vitamin D and NF1: The Mast Cell Connection

In and around 1985, Silveira et al. documented that mast cells sequester calcidiol. [19,20]. They selectively and carefully investigated calcidiol synthesis and fate in a wide variety of mammals, including humans, dogs, cats, rabbits, rats, mice and opossums. The only potentially significant inter-species difference related to the calcidiol-containing granules in KC appearing "coarse" in the opossum and "thin" in all other species. In any event, they clearly established that some portion of KC-synthesized calcidiol does not enter the blood stream directly, but rather is ingested by resident mast cells and incorporated into mast cell granules, presumably for discharge (paracrine secretion) elsewhere. Respecting this set of facts in the context of the well-known importance of mast cells in NF1, one must immediately query whether the absolute number or density of skin

mast cells significantly influences the timing and amount of calcitriol available for conversion to calcidiol and calcitriol – especially in circumstances where the number of cutaneous mast cells may be increased or decreased. For example, are the number and density of skin mast cells in NF1 and the various forms of mastocytosis determinants of vitamin D homeostasis in affected individuals? More specifically, is calcitriol sequestration by skin mast cells an important element of human NF1 pathogenesis?

In addition to concerns about mast cell influences on calcitriol production and availability, there is also the obverse concern: does calcitriol influence mast cells? In 1996, Toyota et al. [21], using mouse peritoneal mast cells and a human mast cell line (MC/9), showed that calcitriol inhibits mast cell proliferation and decreases A23187-induced histamine release. They suggested that these effects might be associated with a decrease in mast cell expression of c-kit. In 1999, Nakayama et al. [22] applied vitamin D3 analogues to supposed café-au-lait spots (CLS) of persons with NF1. In one instance, small portions of an NF1 CLS were explanted to the skin of nude mice and a 22-oxacalcitriol ointment applied twice daily for 14 days. The treated segments “showed something like incontinence of pigment ... loss of melanin from basal layer cells, with accumulation of the melanin in the upper dermis within melanophages.” Bromdeoxyuridine labeling of three treated segments (compared to three untreated segments) showed fewer labeled cells (4.2 ± 0.4 versus 38.0 ± 2.4 , $p < 0.01$). In a second instance, one half of a 4 cm x 6 cm NF1 CLS was treated in situ with a tacalcitol ointment (twice daily for 4 weeks). Histologically, “the treated portion showed an obvious decrease of melanin in the basal layer of the epidermis compared with the untreated portion.” In a third scenario, a “CLS” involving “the entire back” of a 6-year-old child was treated twice daily with tacalcitol ointment for 6 months. Photographs before and after treatment were used for assessing outcome. The published figure “shows a clear decrease in the pigmentation of a large pigmented macule ... after only 3 months of treatment. Effects ... continued for up to one year and a half without recurring hyperpigmentation.” In this third protocol, the targeted lesion was probably not a CLS, but rather hyperpigmentation overlying a diffuse plexiform neurofibroma. In any event, my intent presently is not to endorse the conclusions, but rather to draw attention to the opportunities for assessing the interplay of vitamin D congeners and analogues on the one hand, and various cell types important in NF1 on the other hand, including mast cells and melanocytes.

The presence of excess numbers of mast cells in NF1 neurofibromas has been known for decades, although the details of the behavior and capabilities and origin of these neurofibroma mast cells have not been studied. In 1993, Hirota et al. [23] studied the density of mast cells in human neurofibromas and compared those densities to mast cell densities in non-NF1 skin. In five human neurofibromas, the mast cell density was $464/\text{mm}^2$ (range 312-660), while in non-NF1 normal skin mast cell density was $66/\text{mm}^2$ (range 40-96). The authors’ data on c-kit and kit-ligand expression in the neurofibromas and mast cells were consistent with the high mast cell density in the neurofibromas and compatible with other investigations exploring these relationships [24,25].

Consistent with the documentation of high mast cell density in one type of NF1 lesion-neurofibromas-Okazaki et al. [26] documented increased mast cell densities in NF1 CLS ($140/\text{mm}^2$) and non-CLS NF1 skin ($50/\text{mm}^2$) compared to non-NF1 non-CLS (control) skin ($15/\text{mm}^2$). Three years later, in 2006, De Schepper et al. [27] examined

the mast cell density in four types of specimens: NF1 CLS, NF1 “normal” skin, control subject CLS and control subject “normal” skin. The respective mast cell densities (extrapolated from bar graph data) were $58.8/\text{mm}^2$, $66.0/\text{mm}^2$, $61.2/\text{mm}^2$ and $32.8/\text{mm}^2$, with $p < 0.05$ for all comparisons. The over-arching point here is that NF1 skin is replete with excessive numbers of mast cells, even before considering the excess numbers of mast cells in neurofibromas, including cutaneous neurofibromas strictu sensu.

In this regard, and adding to the data of Hirota et al. [23] Lammert et al. [7] not only confirmed the excess of mast cells in NF1 neurofibromas, they established a highly significant inverse relationship between cutaneous neurofibroma number (a very reliable proxy for mast cell numbers/densities) and serum calcidiol concentration. Cutaneous neurofibroma numbers (per person) were estimated and counted to afford placement in either of three categories: 10-99, 100-999 or >1000. After assiduously adjusting for time of year (sun exposure) and diet, the mean serum calcidiol was 14.0 ± 1.6 ng/ml for persons with NF1 and 31.4 ± 1.7 ng/ml for the control group, with $p = 0.0001$. The inverse correlation between serum calcidiol and number of cutaneous neurofibromas was highly reliable, with $p = 0.00001$.

The sum of published data to this point at the least is compatible with the hypothesis that the NF1 neurofibroma burden (numbers and sizes of cutaneous neurofibromas and/or numbers and sizes of plexiform neurofibromas) accounts for the deficit in circulating vitamin D levels in persons with NF1. In at least some sense then, perhaps it is useful to construe NF1 as a distinctive type of mastocytosis. I am explicitly suggesting that in some very compelling sense, NF1 can be considered a Schwann-cell-enhanced mastocytosis. Among the consequences of such a thesis are the opportunity to account for additional NF1-related abnormalities in terms of excess numbers and abnormal locations of mast cells and the potential for co-opting mastocytosis treatment strategies.

In this context I would point out that there have been several reports of treating systemic mastocytosis and mastocytomas with mast cell blockers, including ketotifen, for which there is already substantial experience in treating persons with NF1 [4,28,29]. The case report of Graves et al [30] is especially relevant in this setting. In 1990, they reported on a 59-year-old man with systemic mastocytosis and extensive skeletal involvement, including bone-associated pain. Prior to treatment, his serum calcitriol was low at 12.0 pg/ml (normal 15-50 pg/ml), while his serum calcidiol was not low at 37.6 ng/ml (normal 15-45 ng/ml). This discrepancy in the baseline levels of serum calcidiol and calcitriol levels is noteworthy: reliance on the calcidiol measurement alone might well have led to the wrong conclusions about his vitamin D and mast cell dynamics. At the same time, the patient’s serum osteocalcin was also low at 1.0 ng/ml (normal 1.8-6.6 ng/ml), and his histamine level was high in plasma at 3.7 ng/ml (normal <1.0 ng/ml) and high in urine at 68.7 $\mu\text{g}/24$ hrs (normal <50 $\mu\text{g}/24$ hrs). Treatment with ketotifen, 1 mg twice a day for six months, led to normalization of his serum calcitriol (21.5 pg/ml), serum osteocalcin (3.0 ng/ml) and urinary histamine (19.0 $\mu\text{g}/24$ hrs). After three months of treatment, his plasma histamine was normal (<1.0 ng/ml), but it was “slightly elevated” at six months (1.68 ng/ml). “Bone density measurement at 6 and 14 months of therapy showed no further bone loss.” Bone biopsy after 6 months of ketotifen treatment showed a “reversal of evidence of bone loss.” The authors concluded that “[t]he use of ketotifen resulted in improvement in bone pain, as well as symptoms of pruritus, flushing, constitutional symptoms and

gastrointestinal symptoms.” Ketotifen as a treatment approach for systemic mastocytosis is not unique [31].

Mast Cells and NF1: A Role for Mast Cell Blockers?

At the least, the literature cited here and elsewhere emphasizes that there is substantial heterogeneity among mast cells in terms of sites of origin, maturation and performance. Mast cells from the skin appear to involve vitamin D metabolism in ways that mast cells originating in the peritoneum or bone marrow cannot. We need to know the origin and history of mast cells in NF1 neurofibromas. Moreover, mast cells in NF1 are important well beyond neurofibroma initiation and growth. They also obviously influence vitamin D dynamics, neurological performance (including cerebral function), bone tissue dynamics, wound healing, anticoagulant function and small vessel hemorrhage. Treatments designed to decrease mast cell function likely can do much more than simply decrease the rate of neurofibroma initiation and/or neurofibroma growth. In addition to the obvious potential for improving vitamin D dynamics and skeletal health, the opportunity to minimize intra-operative small-vessel hemorrhage is cogent [4].

It has been almost three decades since I began promoting the use of ketotifen in the treatment of NF1 [4,28,29]. Others have corroborated ketotifen's NF1-related efficacy and potential [32]. Now, with the compelling connections afforded by this literature review, there is an urgent need to consider immediately protocols exploiting overwhelming potential benefits commensurate with the low cost and minimal risks of an extraordinarily benign drug with which thousands of clinicians are already familiar.

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